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Case report

DNA analysis of skeletal tissue recovered from the English Channel

Jamie Fredericks, PhD, MSc, Post-doctoral Research Scientist [a,*],
Kaia Brown, MSc, BSc (Hons), MSc Student [a],
Anna Williams, PhD, MSc, Head of Forensic Anthropology Research [a],
Phil Bennett, PhD, Clinical Scientist [b]

[a] Cranfield Forensic Institute, Cranfield University, Shrivenham SN6 8LA, UK
[b] Micropathology Limited, The Venture Centre, University of Warwick Science Park, Coventry CV4 7EZ, West Midlands, UK

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ABSTRACT

This case study reports on DNA analysis from different skeletal elements of an individual recovered 2 years after going missing in the English Channel. Selected bones were also re-submerged for an additional 2 years and re-analysed. By comparing results from both time points, this study concludes that high-load-bearing bones, particularly the bones of the feet, appear to be a preferable source of DNA for identification of skeletal tissue recovered from similar conditions.

1. Introduction

The extraction and subsequent analysis of DNA from skeletal tissue have played a pivotal role in the identification of human remains in mass fatality incidents, such as the 2011 World Trade Center disaster,[1] the 2004 tsunami in Southeast Asia[2] and military conflicts.[3] As such, the continued development of increasingly streamlined, efficient and cost-effective laboratory procedures for DNA analysis using degraded skeletal remains is important.[3] This includes methods for identifying the best sources of potentially viable DNA from the total set of available remains. Recent studies have suggested that the success rate of identification is greater when using DNA extracted from bones of the feet, rather than from the traditionally used high-load-bearing long bones.[1,4]

The current study was performed using skeletal elements obtained from a Caucasian male whose remains were recovered approximately 2 years after being reported missing in the English channel. The remains were discovered on the seabed only a few hundred metres from the location where the individual was last seen. Consequently, it was assumed that the remains had been continuously submerged for the duration of the period in which the individual was missing.

After initial analysis of DNA short-tandem repeat (STR) profiles from the range of different skeletal elements available, selected bones were re-submerged for a further 2 years. The aim of this was to investigate any differences in the success/failure rate of DNA profiling on varied skeletal elements that had been submerged over prolonged periods and hence to determine whether the use of any specific bones may be preferable when several types are available.

2. Materials and methods

2.1. Re-submersion of skeletal tissue

Skeletal elements for re-submersion were selected based upon the amount of original material that was available. They were re-submerged in a tank containing synthetic Instant Ocean Seawater (Spectrum Brands Inc., Madison, WI, USA). The tank was kept in a fridge and the water maintained at an average temperature of 8 °C and pH 7.5. The salt concentration was maintained at 34 ppt using a hydrometer and the entire water volume was replaced monthly.

2.2. DNA extraction from bone

DNA was extracted from 500 mg of demineralised bone using the Genial®: First DNA All-tissue DNA-extraction kit (GEN-IAL GmbH, Troisdorf, Germany). The protocol was as detailed by the

* Corresponding author. Tel.: +44 (0) 2476 323222; fax: +44 (0) 2476 323333.
E-mail addresses: jfredericks@hotmail.co.uk (J. Fredericks), a.willaims@cranfield.ac.uk (A. Williams), p.bennett@micropathology.com (P. Bennett).

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Table 1
The number of PowerPlex® 16 loci successfully amplified from skeletal elements that had been submerged in the English Channel for approximately two years.

	PowerPlex ® 16 STR Loci (bp size range)																													
	D3S1358		D5S818		Vwa		THO1		D13S317		D21S11		D8S1179		D7S820		TPOX		D16S539		D18S51		CSF1PO		FGA		Penta D		Penta E	
Bone	(115-147)		(119-155)		(123-171)		(156-195)		(176-208)		(203-259)		(203-247)		(215-247)		(262-290)		(264-304)		(290-366)		(321-357)		(322-444)		(376-449)		(379-474)	
Clavicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	+	-
Calcaneus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Femur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibula	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Intermediate Cuneiforms	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lumbar Body	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lumbar Process	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lateral Cuneiforms	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Medial Cuneiforms	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Metatarsals	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Navicular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pelvis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
Radius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rib	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-
Sacrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Scapula	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Talus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thoracic Body	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thoracic Process	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tibia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Ulna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

manufacturer save one modification. To reduce both the risk of inter-sample contamination and the potential for heat-induced DNA damage, thin shavings of bone (instead of bone powder obtained by drilling, as described in the manufacturer's protocol) were used. These were obtained by removing the outer surface of bone that had been demineralised in 500 mM ethylenediaminetetraacetic acid pH 7.5 (exchanged daily) for 5 days at 8 °C, using a disposable scalpel. The resulting DNA pellet was re-suspended in 60 μl of DNase- and RNase-free water.

2.3. STR profiling

DNA from each skeletal element was genotyped using the PowerPlex® 16 HS Human Genotyping Kit (Promega, Southampton, UK), which targets 15 independent microsatellite loci and amelogenin. The protocol was as detailed by the manufacturer; each polymerase chain reaction (PCR) reaction contained 10 μl of template DNA and underwent 32 thermal cycles. Analysis of the PCR products was performed using an ABI 310 genetic analyser (Applied Biosystems, Cheshire, UK).

3. Results

After approximately 2 years of submersion in the English Channel, it was possible to obtain consistent full (i.e., all 15 autosomal STR loci) profiles from 13 of the 20 bones recovered. These data were used to define an original consensus profile, which was assumed to be that which would have been obtained from fresh material from this individual whilst alive. Locus and/or allele dropout occurred in the clavicle, humerus, pelvis, sacrum, tibia, fibula and rib, whilst no markers could be amplified from either the radius or the ulna (Table 1).

Locus and allele dropout were observed in all bones that underwent submersion for an additional 2 years (approximately 4 years in total). This occurred most notably in the tibia and fibula, 14 loci having been obtained originally, with this declining to two and zero, respectively, after an additional 2 years. However, better preservation of high-molecular-weight STR amplicons was observed from DNA extracted from the femur and foot bones. This was particularly apparent for the lateral and intermediate cuniforms, where the largest STR loci (in terms of

Table 2
The number of PowerPlex® 16 loci successfully amplified from skeletal elements that had been submerged for approximately four years; two years in the English Channel and two years in synthetic seawater.

	PowerPlex ® 16 STR Loci (bp size range)																													
	D3S1358		D5S818		Vwa		THO1		D13S317		D21S11		D8S1179		D7S820		TPOX		D16S539		D18S51		CSF1PO		FGA		Penta D		Penta E	
Bone	(115-147)		(119-155)		(123-171)		(156-195)		(176-208)		(203-259)		(203-247)		(215-247)		(262-290)		(264-304)		(290-366)		(321-357)		(322-444)		(376-449)		(379-474)	
Calcaneus	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-
Femur	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-
Fibula	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Intermediate Cuneiforms	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
Lateral Cuneiforms	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
Medial Cuneiforms	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
Metatarsals	-	+	+	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
Navicular	-	-	+	+	-	+	-	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	-	+	+	+	-	-	-
Radius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rib	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Talus	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	-
Tibia	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

PCR product length), Penta D and Penta E, were successfully amplified (Table 2).

Locus dropout was usually the result of the unsuccessful amplification of the high-molecular-weight loci, including D18S51, CSF1PO, FGA and Penta D and E. When high-molecular-weight loci dropout was seen in a sample, loss of heterozygosity in low-molecular-weight markers was also usually recorded.

The amplification of a 500-base-pair fragment of the human *c-myc* gene (for details see reference 5) was not noticeably impaired when high-quality control DNA was diluted in DNA extracted from selected bone samples, as compared with control DNA similarly diluted in Molecular Grade water (data not shown). This suggested that it was unlikely that the observed results were due only to the increased presence of PCR inhibitors, as opposed to actual DNA degradation.

4. Discussion

The results of this study are consistent with previous reports that have shown it possible to obtain a usable DNA profile from bones submerged in water for an extended period of time.[6–8] Through the re-submersion of a number of skeletal elements, we have shown that the preferred choice of bone for DNA analysis may be influenced by the duration of submersion. Moreover, the results suggest that for each skeletal element in the study, the rate of DNA degradation is different. Therefore, the 'end point' at which no DNA can be amplified could be bone dependent. After a 4-year period in seawater, the greatest numbers of loci amplified came from DNA extracted from foot bones. Another factor of note was the high incidence of allele dropout in 'low-load'-bearing bones, evidenced by loss of heterozygosity at certain loci, in profiles showing complete allele loss at other loci. The effects that this may have on the ability to reliably confirm or exclude identity should be considered in all such cases. The results show that DNA was better preserved in 'high-load'-bearing bones, suggesting that the 'end point' for skeletal elements, such as the femur and foot bones, may be longer than that of 'low-load'-bearing bones.

The use of 'high-load'-bearing bones, including those in the foot, has been particularly useful when identifying victims of mass disasters such as the Asia Tsunami. The percentage of individuals that were successfully identified from the World Trade Center terrorist attacks was highest when using DNA extracted from bones including metatarsals and phalanges.[1] Although load bearing, these skeletal elements have a higher ratio of cancellous to compact bone, when compared to long bones such as the femur. It is thought that cancellous bone may have a higher cell content than compact bone due to increased vascularisation.[4]

5. Conclusion

Traditionally, DNA is often extracted from the femur when identifying skeletal remains. In light of this case study, DNA analysts, pathologists and anthropologists should be aware that successful identification of compromised skeletal remains, such as those in this case study, may come from extracting DNA from an unorthodox choice of bone, such as foot bones. When selecting the skeletal element for analysis, the environmental conditions from which remains are retrieved should also be taken into account. This case study represents skeletal remains recovered from cold water; it can only be assumed that the rate of DNA degradation would be greater in warmer waters, such as the Indian Ocean. Given the success rate of STR profiling and the relative ease of removing bone elements from the foot, it may be preferable to select these 'high-load'-bearing bones for DNA analysis. This would ultimately save, time, money and effort in identifying individuals from skeletal remains.

Ethical approval

The Cranfield University Health Ethics Committee granted ethical approval for this study and informed consent was obtained from the individual's next of kin in conjunction with Her Majesty's Coroner, from whose custody the remains were released.

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None.

Conflict of interest

None.

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